LECTIN ACTIVITY OF CHROMATIN NON-HISTONE PROTEINS

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Abstract. Non-histone proteins from chromatin of sea urchin embryos were found to possess the ability to agglutinate erythrocytes.

I. INTRODUCTION

Early in this century a class of plant proteins or glycoproteins able to agglutinate erythrocytes was discovered and designed as lectins. Recently, developmentally regulated lectins have been identified in different species of the cellular slime mold [1–4], as well as in tissues of Electrophorus electricus [5]. Nowak et al. [6] have reported the presence of developmentally regulated lectin in extracts of embryonic chick muscle and of the differentiating myoblast clone L6.

We have found previously [7] that non-histone proteins of chromatin from sea urchin embryos contain glycoprotein components. Evidence that several molecular weight classes of chromosomal proteins are glycoproteins was recently presented by Stein et al., [8]. In current experiments devoted to this subject non-histone proteins were assayed for erythroagglutination as an essential lectin activity. In this paper we wish to present evidence that non-histone proteins of sea urchin embryos do contain lectin-like component(s).

II. MATERIALS AND METHODS

Eggs of sea urchins P. lividus were fertilized and embryos harvested at the blastula, gastrula and pluteus stage. Nuclei were prepared by homogenization of embryos in 0.075 M NaCl, 0.025 M EDTA, pH 8.0, followed by centrifugation of the homogenate at 15000× g. Crude nuclei were treated with an inhibitor of protease activity (0.5 mM phenyl-methyl-sulphonyl-fluoride) and their membrane was stripped off by washing with 0.43% Na-deoxycholate plus 0.86% Tween 40. Crude chromatin was washed several times with 0.01 M Tris buffer, pH 8.0, and purified by sedimentation through 1.7 M sucrose. Chromatin preparations were
dissociated in 1 mM phosphate buffer, pH 6.8, containing 2 M NaCl, 5 M urea, sonicated and submitted to chromatography on hydroxyapatite columns as reported before [9]. The non-histone proteins eluted from the column with 0.05 M Na-phosphate buffer, at pH 6.8, containing 2 M NaCl and 5 M urea, were dialyzed against distilled water and lyophilized.

Agglutination activity of the non-histone proteins was assayed in the system containing fresh or trypsinized erythrocytes. Cells were prepared from fresh blood collected in Alsever's medium that had been washed 4 times with 0.15 M NaCl. To obtain trypsinized erythrocytes, a 4% suspension of fresh cells in 0.1 M Na-phosphate, pH 7.4, 0.05 M NaCl, containing 1 mg ml⁻¹ of trypsin, was incubated at 37 °C for 1 hr. The cells were then washed 4 times with 0.15 M NaCl, and suspended in a medium containing 0.075 M NaK-phosphate, pH 7.2, and 0.075 M NaCl (indicated by PBS). Agglutination assays were performed using microtiter V plates. The lyophilized non-histone proteins hardly dissolved in PBS. Solubilization was improved by short heating at 60-70 °C. For agglutination assays two-fold diluted non-histones or Con A, 0.025 ml of PBS and 0.025 ml of a 4% suspension of trypsinized or untreated sheep erythrocytes were mixed. In certain series the PBS solution was replaced by 0.025 ml of 1% bovine serum albumin in PBS or by 0.025 ml of the sugar in question used for hapten inhibition studies. After shaking, the mixtures were incubated at 37 °C for 1 hr and kept overnight at 4 °C.

III. RESULTS AND DISCUSSION

Both untreated and trypsinized sheep erythrocytes were agglutinated by the non-histone proteins, the effect being markedly higher with trypsinized cells. As shown on Figure 1, unagglutinated erythrocytes formed a clear dot on the bottom of the well, whereas agglutinated cells clamped into more or less large aggregates. The agglutination activity of the non-histone proteins from the blastula stage was higher than that of the gastrula stage, whereas the activity of the pluteus sample was lowest. However, even the activity of the blastula non-histones was, in comparison to that of Con A, rather low. Agglutination activity of solutions with a titer as low as 1 (1 mg of protein per ml) corresponded to the activity of Con A with a titer of 1:300. The highest titer of the blastula, the gastrula and the pluteus non-histones that still agglutinated the cells were 1:16, 1:8 and 1:4, respectively. The low agglutination activity of the non-histone proteins might be explained either by a low agglutinin content, or by developmentally regulated blockage of sites which bind to specific saccharide groups on the surface membrane. D-glucose inhibited the activity of Con A, but not that of the non-histone proteins. While alpha-lactose in 0.1 M concentration had an effect upon the activity of neither Con A nor on non-histone protein, D-galactose diminished the activity of the non-histones, but not that of Con A. These findings indicate that the binding sizes of Con A and of agglutinin constituent(s) of non-histone proteins may be different.

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266